2024 Summer Scholar Profile: Alice Zhang

My name is Alice Zhang, and I am a rising senior at Wellesley College majoring in Data Science with a concentration in Bioinformatics. At school, I work in the Kellis Lab under Professor Manolis Kellis, where I research inclusive polygenic risk score models (iPGS) and the systematic application to brain MRI-derived traits with my mentor Dr. Yosuke Tanigawa. My project involves using iPGS to analyze brain MRI-derived traits, such as brain volumetric measurements. The project is scaling up across many brain MRI-derived traits, integrating with brain eQTL datasets, and modeling multiple response variables/brain MRI-derived traits simultaneously. At the Buck Institute, I interned in the Schilling Laboratory under Professor Birgit Schilling with my mentors Dr. Joanna Bons and Dr. Mark Watson. The Schilling Lab studies the molecular mechanisms involved in aging and age-related diseases through uncovering protein signatures and pathways using mass spectrometric technologies.

My first project was developing a protocol to help researchers generate data-dependent acquisition (DDA) protein posttranslational modification (PTM) spectral libraries for proteome-wide lysine succinylation analysis of data-independent acquisition (DIA) data. DIA simultaneously collects fragment ion spectra for all detectable precursor ions isolated within a defined wide m/z range, generating highly complex spectra with fragment ions from multiple co-eluting peptides and allowing for comprehensive, reproducible, and unbiased quantitative proteome analysis. Deconvoluting DIA MS/MS spectra is, however, computationally intensive, requiring complex, sophisticated and dedicated analysis tools. One approach is to use the information contained in a spectral library generated from DDA data to perform DIA data extraction, peak group scoring, and PTM site localization probability determination for peptide and PTM identification and quantification. DDA relies on iteratively selecting precursor ions for fragmentation based on predefined criteria, typically ions with the strongest signals from the survey scans. Collected DDA MS/MS spectra are then submitted to database search for identification. A spectral library generated from high-quality DDA data contains experimentally observed MS/MS spectra matched to peptide sequences with high confidence, leading to high identification confidence, sensitive and accurate quantification, and enhanced probabilities of correctly identifying and accurately localizing the modifications, which is necessary to understand their biology. Using DDA data collected on mouse brain succinyl enrichments, we generated spectral libraries in Spectronaut (Biognosys), Spectromine (Biognosys), Proteome Discoverer (Thermo Fisher Scientific) and MSFragger through FragPipe (Nesvizhskii Lab), which are all search engines, applying finely optimized settings. We reported our results and conclusions in the protocol paper, “Generating High-Quality Data-Dependent Acquisition Succinyl Spectral Libraries for Proteome-wide Succinylome Analysis by Data-Independent Acquisition” to be submitted in *Methods in Molecular Biology*.

Cellular senescence is hypothesized to be involved in aging and many age-related diseases. The Schilling laboratory is very collaborative, and my second project was in collaboration with the Campisi laboratory at the Buck Institute, working on the R Shiny app FAST.R. FAST.R is part of the Fully-Automated Senescence Test (FAST) workflow developed by Dr. Francesco Neri that allows for a relatively simple way of automated detection of cellular senescence (Neri *et al.*, *GeroScience*, 2024). Because there is no universal marker for senescence, it is typically determined manually through looking at several senescence associated markers. FAST was developed and optimized to automatically and rapidly detect senescence in cell cultures with a predefined set of three markers; however, we wanted to make FAST compatible with any cell types. I spent the second half of my summer recoding FAST.R, adding flexibility to the number and types of markers, and published the new code to GitHub. We have tested this code on cells from the brain and cartilage, demonstrating the flexibility, utility, and ease-of-use of this new iteration of FAST.R. This code will be used in future FAST projects that are being developed in the lab now.

2024 Buck Summer Scholar: Anna Buretta

My name is Anna Buretta, and I am a rising sophomore at Princeton University majoring in neuroscience. I am interested in researching synapse function in neurodegenerative diseases, such as Alzheimer's and Parkinson's disease.

Over my gap year, I worked in Dr. Michael Kaplitt's lab at Weill Cornell Medicine, studying gene therapy to treat Parkinson's disease. Parkinson's disease is characterized by loss of controlled movements, such as tremor, stiffness, and slowed movements. These behaviours are driven by a loss of dopaminergic neurons in the substantia nigra (part of the brain involved in coordinating and producing movement). In the Kaplitt lab, I worked on examining the neuroprotective effect of the Akt/PTEN pathway in dopaminergic neurons using a Parkinsonian mouse model.

This summer, I worked in the Tracy lab, which researches how synapses (the connections between neurons) are disrupted in disease. The lab focuses on how tau, the microtubule-associated protein, can affect memory and synapse health in diseases such as Alzheimer's and Frontal temporal dementia (FTD). In the Tracy lab, I worked under Ph.D. candidate Doyle Lokitiyakul to study molecular mechanisms of memory formation. Memories are encoded in connections between neurons, and the strengthening of neural connections drives memory formation. To strengthen connections, new receptors are trafficked to the post-synapse, sensitizing the post-synaptic neuron to the pre-synaptic neuron. The production of these new receptors relies on a phenomenon called local translation, where free floating mRNAs are translated following external stimuli. In the Tracy lab, I studied a translation initiation factor which we believe to be important in regulating the local translation of new receptors. I examined interactions within the translation initiation complex and other proteins involved in local translation. I also explored the post-translational modifications that regulate the activity of the translation initiation factor. Lastly, I investigated the translation initiation factor's ability to rescue translation of new receptors in FTD neurons. Through the experiments, we hope to characterize the mechanism that the translation initiation factor uses to promote memory formation.

2024 Buck Summer Scholar: Anshuman Das

My name is Anshuman Das, I am a rising senior at Vassar College in Poughkeepsie, New York. At Vassar College, I am majoring in Cognitive Science with a focus on Evolutionary Biology. I am involved in a research project under the guidance of Dr. Lori Newman, where I work to understand the role of astrocytes in synaptic plasticity for spatial working memory in rat models using DREADDs (Designer Receptors Exclusively Activated by Designer Drugs). At the Buck Institute, I am working in the Ellerby Lab under the supervision of Dr. Kenneth Wilson, a postdoctoral fellow, and Dr. Lisa Ellerby. My work in the lab focused on the role of the intercellular transport system and its connection to cellular senescence, which is when cells stop dividing and accumulate in the body. Senescent cells can cause a myriad of irregular functions and lead to the development of neurodegenerative diseases. Senescence has been connected to many neurodegenerative diseases such as Alzheimer's Disease (AD).

The Ellerby Lab is specifically focused on neurodegeneration, which is a type of disease that causes progressive damage to the neural cells. This damage, in turn, has trickle-down effects throughout the rest of the body, sometimes leading to changes in mood, behavior, and cognition. I am particularly interested in the disease models of Alzheimer's, Parkinson’s, and Huntington’s disease. All of these diseases can be attributed in part to the formation of toxic protein clumps. The retromer, one of the many cellular transportation systems, is responsible for the efficient movement of macromolecules around the cell, including proteins. It connects different cellular compartments, or organelles, together. In cases where this transport system breaks down, it can lead to the accumulation of these toxic clumps, which can be disastrous to cell viability. These dysfunctions in the retromer are specifically tied to the onset of neurodegenerative diseases. A component of interest within the transport system is OXR1, which is a structural protein responsible for holding the retromer together. OXR1 is lost in people with age, and the loss of OXR1 has been connected to neurological defects. More specifically, on a gene expression level, loss of OXR1 shows a similar pattern to Alzheimer’s disease. This loss can serve as an indication of DNA damage, which is a hallmark of aging. DNA damage can lead to cellular senescence.

In our project, I wanted to solidify the connection between the retromer and the onset of senescence. Therefore, in my research I am focused on highlighting senescence in the mouse model brains with OXR1 overexpression. I am using human neurons to understand the impact of knocking down OXR1 and also overexpressing it to see if the overexpression would rescue cellular senescence. These experiments would in turn help solidify the connection between senescence and retromer dysfunction.

2024 Buck Summer Scholar: Daniel Allred

My name is Daniel Allred. I am a student at the College of William & Mary, majoring in Biology and minoring in Data Science. At William & Mary, I work in Dr. Matthew Wawersik's lab which focuses on cellular and developmental genetics. Our current research investigates how the gene *Chigno* helps regulate stem cell development in fly testes. During the school year, I have been designing and conducting a transcriptomic analysis of *Chigno* in fly testes. At The Buck Institute, I had the opportunity to work in Dr. Chuankai Zhou's Lab, alongside postdoctoral researcher Dr. Meiying Wu. The Zhou lab focuses on proteostasis and its role in cellular aging, primarily using budding yeast as a model organism. One of the lab's main goals is to develop new methodologies that enable groundbreaking aging research.

My work with Dr. Meiying Wu centered on developing a microfluidic protocol to streamline the quantification of yeast replicative lifespan (RLS). RLS, defined by the number of replications a yeast cell undergoes, is a valuable measure of aging in budding yeast. Replication occurs when one yeast cell, called the mother, creates a new yeast cell, called the daughter. Quantifying RLS is a challenging and time-consuming task because scientists must count every replication of an individual yeast cell by observing that cell under a microscope. The biggest roadblock to quantifying RLS is dealing with the daughter yeast cells, which, after being produced, also start replicating. The average yeast RLS lasts 20 replications. With every replication of the mother and daughter cells the entire population of yeast doubles. Thus, over the RLS of a single yeast millions of cells are produced. This creates a crowded environment, making it difficult to identify individual replications. Previous methods required manual microdissection to remove new cells as they replicated. This prevented exponential growth and allowed for quantification but required significant time and labor. More recently, microfluidic approaches have been developed.

Microfluidics lie at the intersection of physics, engineering, and biology. They use small channels to move small amounts of liquid. Current microfluidic approaches for quantification of yeast RLS use mechanical traps. These traps take advantage of the size difference between mother and daughter yeast, allowing mother cells to be trapped while smaller daughter cells are washed away. Unfortunately, this approach has several limiting factors including restrictive requirements and relatively low sample size. The protocol we've been developing uses chemical adhesion to bind and image yeast. The protocol uses a relatively large channel and coats the surface with proteins. These proteins bind to the yeast and adhere them to the channel's surface. Any daughter cells formed by the first generation of mother cells will not be bound to the protein adhesive. Thus, as liquid flows through this channel providing nutrients and experimental conditions, it will wash away all newly formed daughter cells. This provides an efficient method to quantify yeast RLS. The process of troubleshooting and developing a reliable protocol is long and difficult. However, the finished protocol will provide a platform to conduct a range of new yeast RLS studies, with topics ranging from iron homeostasis to protein behavior.

2024 Buck Summer Scholar: Xinran (Jess) Liu

Hello everyone! My name is Xinran (Jess) Liu. I'm a rising junior majoring in Molecular, Cell, and Developmental Biology and minoring in Neuroscience at the University of California, Los Angeles (UCLA). At UCLA, I work in Dr. Daniel C. Lu's Neuroplasticity & Repair Lab under the guidance of Dr. Ruyi Huang. The lab aims to elucidate the mechanisms behind motor and respiratory functions encoded in the spinal cord using rodent models. We also investigate how neuromodulation on the spinal cord can restore lost functions and translate these findings to clinical trials for spinal cord injury patients. This summer at the Buck Institute, I joined Dr. Eric Verdin's Lab to study the interplay between aging, metabolism, and the immune system. Working with postdoctoral fellow Dr. Jingqi Fang, I am investigating the role of CD38, a major nicotinamide adenine dinucleotide (NAD)-consuming enzyme, in brain aging.

NAD is a crucial coenzyme involved in cellular metabolism. In aging, NAD levels decrease, which is shown to be linked causally to various age-associated diseases, including cancer, metabolic disorders, brain aging, neurodegeneration, and more. Recent research suggests CD38 increases with age and may contribute to the age-related decline in NAD levels. While studies have shown that CD38-removed mice are protected against obesity, metabolic syndromes, and mitochondrial dysfunction during aging, the role of CD38 in the brain has not yet been explored. Our lab has found that CD38 is predominantly expressed in the choroid plexus (ChP) of the brain. The ChP is a critical yet under-researched structure that produces cerebrospinal fluid (CSF) and forms the blood-cerebrospinal fluid barrier (BCSFB) through junctions between epithelial cells. The passive filtration and active transport of blood components via this barrier enable the production of CSF, which is essential for maintaining brain homeostasis, providing immune surveillance, and removing metabolic waste products. Researchers have reported that with aging, there are decreases in barrier integrity and changes in CSF composition, which are correlated with age-related cognitive decline and neurodegenerative disease symptoms. Given the crucial role that ChP plays in brain aging, it is important to investigate how CD38 is involved in this process.

My project aims to explore how CD38 influences ChP-mediated brain aging. Since CD38 levels increase with age and are predominantly expressed in the ChP, we hypothesize that the increased CD38 levels may be one of the causes of ChP functionality impairment with aging, potentially contributing to age-related brain symptoms. To investigate this, we will compare mice with CD38 removed to mice with normal CD38 expression using multi-dimensional techniques. Our goal is to determine if the loss of CD38 has a protective effect on ChP integrity and brain health. If a correlation is found, we will further investigate the underlying mechanisms. Insights into the role of CD38 in brain aging could be crucial for developing therapeutic strategies to protect overall brain health.

2024 Summer Scholar Profile: Seth Ashby

My name is Seth Ashby. I'm a rising senior at the University of Maine studying microbiology. I have spent most of the last two years studying under Dr. Suzanne Angeli, where we research how processes regulating mitochondria can help extend lifespan in a small transparent worm called *C. elegans*. My work in the lab focuses on how activation of stress responses in a regulated manner can increase lifespan by placing the cell in a state of constant awareness. This work may help decipher different mechanisms that regulate aging in humans. At the Buck Institute for Research on Aging, I worked in Dr. Julie Anderson's lab with my mentor Dr. Minna Schmidt on understanding how exercise reduces symptoms related to Parkinson's disease (PD).

PD is the second most common age-related neurological disorder, estimated to affect over 500,000 Americans nationwide. Patients with PD often experience numerous symptoms, including a reduction in movement, difficulty keeping posture, and loss of gait. Patients can often relieve these symptoms through exercising, but this is inherently harder for patients with more severe symptoms. In the Anderson Lab, we are focused on how we can mimic exercise's benefits chemically to help reduce symptoms for patients with PD.

To chemically mimic exercise, Dr. Schmidt and I searched for compounds associated with exercise and benefits regarding brain health in times of distress, specifically exploring lactate. Lactate is a carbohydrate that is upregulated when exercising and helps the body keep its much-needed energy demands in check. We treated a special kind of *C. elegans* which contains synthetically added proteins that are commonly associated with PD's progression in its muscles. These proteins which normally cluster in the brain and gut of patients with PD are contributing factors to the loss of motor functions seen in patients. Using microscopy, we observed how *C. elegans* treated with lactate were different from those that were not in clustering of these proteins. We then explored whether these changes in clustering would be beneficial or non-beneficial to mobility. The results of this project show that lactate may be acting beneficially in reducing symptoms of PD in *C. elegans*, but the mechanism it uses remains unknown. These projects will act as the groundwork for future research on how lactate could be beneficial for PD patients and give preliminary data for future experiments with more complex models such as mice and humans.

2024 Summer Scholar Profile: Sophia Skubic

My name is Sophia Skubic. I am a rising junior majoring in Behavioral Neuroscience with a minor in Spanish at the University of San Diego (USD). At USD, I work in Dr. Jena Hales's lab, investigating the neurobiological causes of Attention Deficit Hyperactivity Disorder, more commonly known as ADHD. The ADHD model rats perform spatial foraging and memory tasks to test their cognitive abilities. We use immunohistochemistry to examine microglia– cellular markers of inflammation in the brain–in order to study their role in ADHD. This summer, I worked with Dr. Parminder Singh, a postdoctoral fellow at the Buck Institute for Research on Aging, in Dr. Pankaj Kapahi's lab. The lab focuses on dietary restriction to extend lifespan and reduce age-related diseases using several different model organisms. Specifically, my project with Dr. Singh aims to see how dieting and lifestyle choices can affect postmenopausal women and what cellular pathways may lead to increased aging post-menopause. He is interested in how men and women age differently and how this can impact age-related diseases such as Alzheimer's, cancer, and obesity.

On average, women live longer than men, but they are also more susceptible to age-related diseases. Research suggests this susceptibility may be due to differences in their reproductive organs and their roles in the body outside of reproduction. Women go through several stages of reproductive decline in their lifetime, which we characterize as menopause. Once the ovaries stop producing estrogen during menopause, women experience a variety of adverse effects. Estrogen supplementation, the current method of treatment, has proven to be problematic, as it can lead to cancer formation. Our work aims to find a better way to address this deficiency that is more upstream of the problem and could pave the way for more effective treatments and a brighter future for women's health. We believe that the problem begins when inter-organ communication between the ovaries and the brain becomes severed or interrupted by menopause. We are focused on studying the hypothalamic region in the brain because of how closely it is related to aging and postmenopausal disorders. The question that this project aimed to answer was, "Are postmenopausal women more at risk for high fat diet (lifestyle) induced obesity?"

We compared four different cohorts of mice, including normal mice fed a standard and a high-fat diet and ovariectomized mice (our post-menopause mimic) fed the standard and high-fat diets to test our hypothesis. Then, we looked at the hypothalamus in the mice's brains to see where the inter-organ communication between the brain and the ovaries could be failing. We focused on microglia, astrocytes, and stem cells--cell types which may drive women's post-menopause vulnerability to obesity. The lab will utilize a drug that inhibits/activates these cells to see if vulnerability to obesity can be decreased by alteration of these cells. In the future, we hope to bring this information to clinical trials and develop a drug that could mitigate some of the adverse effects postmenopausal women experience, such as obesity.

2024 Summer Scholar Profile: Sriram Selvakumaran

My name is Sriram Selvakumaran, I am a third-year student at the University of California San Diego, majoring in Molecular Cell Biology with a minor in Chemistry. I aim to pursue a career in research by obtaining a PhD focusing on the molecular basis of aging, with the goal of developing interventions to improve health span. I work as an undergraduate researcher in Dr Chengbiao Wu's lab, where I study Alzheimer's disease (AD). AD is a neurodegenerative disorder characterized by cognitive decline, with certain genetic mutations serving as prominent risk factors. One such gene is RIN3, and my research focuses on elucidating the mechanism by which RIN3 mutations contribute to AD by utilizing mice that overexpress the RIN3 gene.

As part of the Summer Scholars Program, I worked in Dr. Ashley Webb's lab. The Webb lab focuses on studying the aging brain, particularly the hypothalamus. Often referred to as the brain's master regulator, the hypothalamus controls body homeostasis and survival-related behaviors such as sleep, circadian rhythms, reproduction, hormonal control, and food regulation. The lab previously identified gene expression changes in the individual cell types of the hypothalamus with age and is now interested in investigating the different phenotypes these aging cells exhibit, the mechanisms driving the expression differences, and potential interventions to improve the activity of the aging neurons.

This summer, I collaborated with Dr. Kaitlyn Hajdarovic, a postdoctoral researcher in the Webb lab, to explore the underlying mechanisms driving age-related changes in neuronal subtypes within the hypothalamus. This research is crucial for developing therapeutics that could potentially rejuvenate aging cells. Typically, researchers rely on animal models or human induced pluripotent stem cell (iPSC) models to study these questions, but these models often fall short in accurately replicating aging phenotypes. To address this, we are advancing direct reprogramming technology to create *in vitro* models of aging hypothalamic neurons. This technique involves converting one cell type into another using transcription factors known as pioneer factors. A key aspect of my summer research was validating whether our reprogrammed neurons exhibit aging characteristics. With age, cells generally display increased DNA damage, mitochondrial dysfunction, nuclear protein damage, and heightened expression of inflammatory molecules. To assess these markers, I employed quantitative PCR to analyze indicators of nuclear protein damage, inflammation, and mitochondrial dysfunction, alongside the Comet assay to measure DNA damage. This approach enabled us to confirm whether the reprogrammed neurons retained aged traits.